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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A23L 1/035	A1	(11) International Publication Number: WO 95/30341 (43) International Publication Date: 16 November 1995 (16.11.95)

(21) International Application Number: PCT/GB95/00921

(22) International Filing Date: 24 April 1995 (24.04.95)

(30) Priority Data:
109614 10 May 1994 (10.05.94) IL

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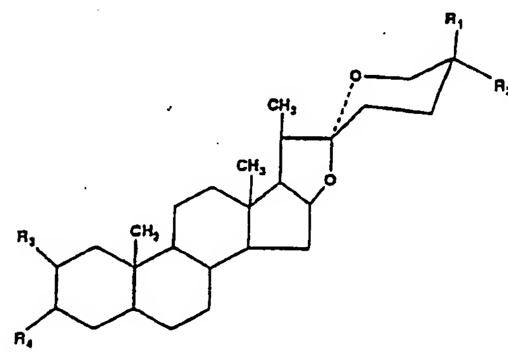
(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

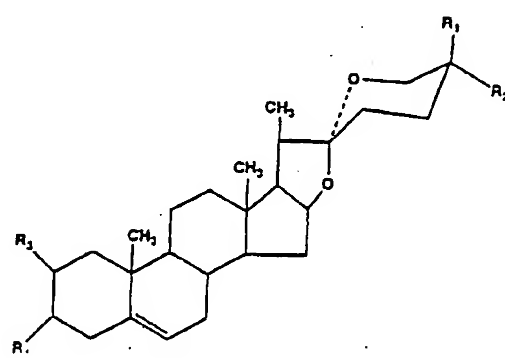
(54) Title: COMESTIBLE PRODUCTS CONTAINING SAPONINS

(57) Abstract

The invention provides a comestible product comprising a fenugreek saponin as an emulsifying agent therein, said fenugreek saponin comprising a mixture of glucosidic steroids having formula (Ia or Ib) wherein R_1, R_2 and R_3 are H, CH_3, H; CH_3, H, H; H, CH_3, OH; or CH_3, H, H respectively; and R_4 is hydrogen or a unit of 2-5 interconnected sugar moieties, the moieties comprising mannose, glucose, rhamanose, xylose and galactose, and having different and improved properties when compared to products produced with soybean saponins.



(Ia)



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COMESTIBLE PRODUCTS CONTAINING SAPONINS

The present invention relates to comestible products. More particularly, the present invention relates to comestible products containing a saponin.

Japanese Patent 1,176,442 (CA112(22):204453r) describes an oil-in-water emulsion useful in cosmetic, food, and pharmaceutical preparations, prepared by heating a hydrogenated lecithin, saponin and polyhydric alcohol, dissolving them, and slowly adding an oil with constant stirring, followed by water, to form a stable emulsion. Thus, a stable emulsion consisting of 3.0 wt.% hydrogenated lecithin A, 0.6 wt.% saponin A, 60.0 wt.% glycerin, and 36.4 wt.% squalane, is prepared.

Japanese Patent 2,258,044 (CA114(9):80302k) describes the preparation of an emulsion prepared by mixing an oil phase containing phosphatidylcholine or phosphatidylethanolamine and an aqueous phase containing saponins. The emulsion is useful in food, cosmetic, and pharmaceutical preparations. For example, 0.3 wt.% phosphatidylcholine was added to 300 g hydrogenated soybean oil, mixed with 700 g defatted milk containing 0.1 wt.% soybean saponins and 0.1 wt.% milk flavor, and homogenized to give a cream beverage.

Thus, the use of saponins from lecithin or soybean origin as emulsifying agents for use in food products is described in the literature.

The name "saponin" is given to substances whose properties have a certain analogy with those of soap: i.e.,

they have the ability to lower the surface tension of water, to foam, and to emulsify oils.

Saponins are sterols or triterpene glycosides [J.E. Courtois, et al., "Complex Glycosides," in The Carbohydrates Chemistry Biochemistry, 2nd Ed., Vol. IIA, W. Pigman and D. Horton, Eds., Academic Press, New York, pp. 213-240 (1970)], mainly of plant origin [E. Heftmann, "C_{27} Saponin and Alkaloids," in Steroid Biochemistry, Academic Press, New York, pp. 37-49 (1970)], and occurring in a variety of plants. Twenty-eight plant species containing saponins have been mentioned in Oxford Book of Plants [Oxford University Press, London (1969)]; the most significant sources of saponins in foods are legumes, such as soybeans, chick peas, peanuts and lentils. Saponins are non-toxic to human beings and are known to lower plasma cholesterol concentrations [H.A. Newman, et al., "Dietary Saponin, A Factor Which May Reduce Liver and Serum Cholesterol Levels," Poultry Sci., Vol. 37, pp. 42-45 (1957); J. Molgaard, et al., "Alfalfa Seeds Lower Low Density Lipoprotein Cholesterol and Apolipoprotein B Concentrations in Patients with Type II Hyperlipoproteinemia," Arthrosclerosis, Vol. 65, pp. 173-179 (1987)]. It has therefore been recommended to consume food rich in saponins to reduce the risk of heart disease [P.R. Cheeke, "Nutritional and Physiological Properties of Saponins," Nutrition Reports International, Vol. 13, pp. 360-365 (1976)].

In a recent review article on saponins in food, the chemical, physical and physiological properties of various saponins are discussed [D. Oakenfull, "Saponins in Food - A Review," Food Chemistry, Vol. 6, pp. 19-40 (1981)].

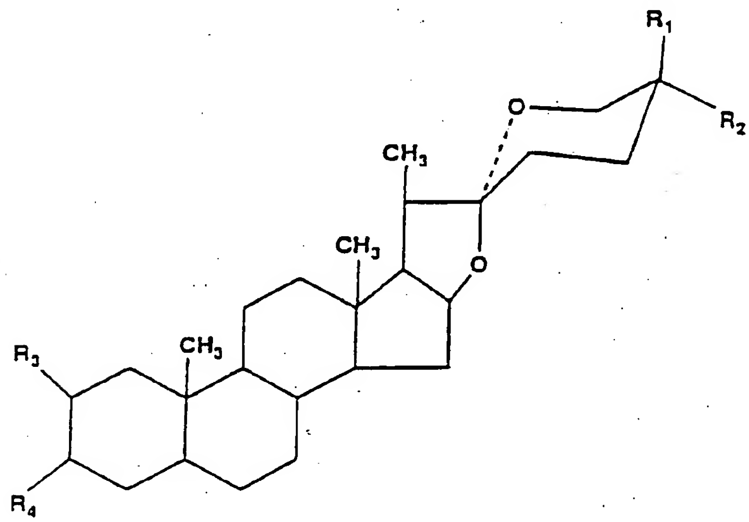
The isolation of saponins from plant materials by extraction is quite tedious and requires the use of organic solvents such as hexane or diethyl ether to remove the lipids, followed by the use of methanol to give the crude saponins [D. Oakenfull, "Saponins in Food - A Review," Food Chemistry, Vol. 6, pp. 19-40 (1981)]. Further purification is difficult, and was achieved by complexing the saponin with cholesterol. The removal of color and odor components requires further treatment.

The foaming power and emulsifying properties of soy saponins, and their possible use as naturally-occuring foaming agents, have drawn the attention of many investigators, and many patents have been registered thereon [for example, F.S. Rostler, et al., "Emulsions for Treating Asphalt Structures," U.S. Patent No. 3,766,107]. It is quite clear from these patents that saponins have potential in the food, cosmetics and pharmaceutical industries [A. Jacques, et al., "Stable Aqueous Fabric Softening Composition Based on Lecithin, Saponin and Sorbic Acid," U.S. Patent No. 4,808,320]. However, few scientific studies are available.

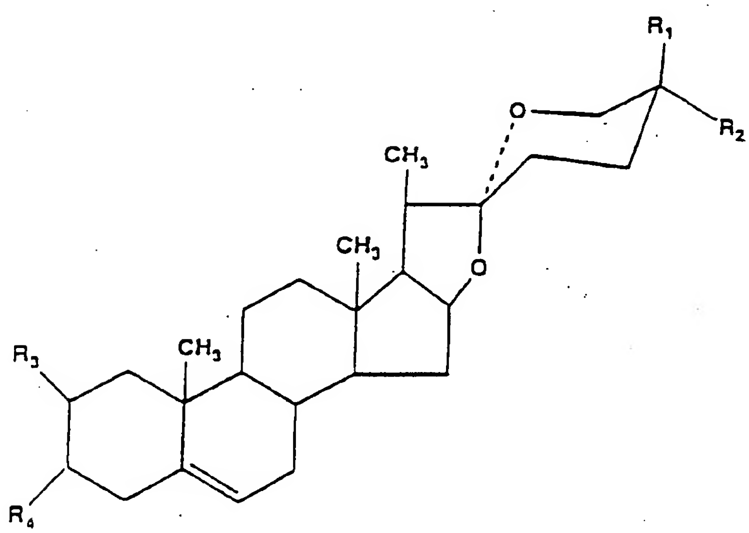
Ruyssen and Loos ["Properties of Saponins, Surface Activity and Degree of Dispersion," J. Colloid. Sci., Vol. 2, pp. 429-451 (1947)] carried out a study on four selected saponins: sapoalbin (the acid form), extracted from the roots of the Gypsophilia species; senegin, which was prepared from the roots of Polyagola senega; digitonin, obtained from Digitoninum cryst; and blighiin, extracted from the fruit shell of the Congolese plant Bliighi Laurentii. It was clearly seen that surface tensions were lowered to values in the range of 55 to 42 dyn/cm. The surface area for sapoalbin was 0.569 nm^2. The interfacial

tension of water/benzene was reduced to about 5 dyn/cm. The emulsification ability was not studied.

According to the present invention, there is now provided a comestible product comprising a fenugreek saponin as an emulsifying agent therein, said fenugreek saponin comprising a mixture of glucosidic steroids having the formula Ia or Ib:



Ia



Ib

wherein:

R_1, R_2 and R_3 are H, CH_3, H; CH_3, H, H; H, CH_3, OH; or CH_3, H, H, respectively; and R_4 is hydrogen or a unit of 2-5 interconnected sugar moieties, said moieties comprising mannose, glucose, rhamanose, xylose and galactose, and having different and improved properties when compared to products produced with soybean saponins.

Saponin from fenugreek grown in India was found to contain 5-6% steroid sapogenins, which percent is subdivided and composed of tigogenin (10.2%), neotigogenin (7.8%), diosgenin (40.3%), yamogenin (16.1%) and gitogenin (12%), together with some other sapongenins such as yuccagenin, neogitogenin and lilagenin, which were present in minor percentages. These sapogeninic structures are quite different from those of soy saponins.

Thus, a first class of preferred embodiments of the present invention is the sapogenins of formula Ia, selected from the group consisting of tigogenin, wherein R_1 is hydrogen; R_2 is methyl; R_3 is hydrogen and R_4 includes 2-glucose, 2-galactose and 1-xylose; neotigogenin, wherein R_1 is methyl; R_2 and R_3 are both hydrogen, and R_4 includes 2-xylose, 1-glucose and 1-rhamanose; gitogenin, wherein R_1 is hydrogen; R_2 is methyl, R_3 is hydroxy and R_4 includes 2-galactose, 1-xyloze and 1-glucose; and neogitogenin, wherein R_1 is methyl and R_2, R_3 and R_4 are each hydrogen.

A second class of preferred embodiments of the present invention is the sapogenins of formula Ib, selected from the group consisting of diosgenin, wherein R_1 is hydrogen; R_2 is methyl; R_3 is hydrogen and R_4 includes 1-mannose, 1-glucose, and 1-rhamanose; yamogenin, wherein R_1 is methyl; R_2 and R_3 are both hydrogen, and R_4 includes 2-rhamanose and

2-glucose; yucagenin, wherein R_1 is hydrogen; R_2 is methyl, R_3 is hydroxy; and lilagenin, wherein R_1 is methyl and R_2, R_3 and R_4 are each hydrogen.

As is known, saponins are amphiphilic compounds in which the polar hexoses are linked to a non-polar steroid group (the sapogenin). The nature of the saponin dominates its physical properties: it is strongly surface active, reducing water surface tension when 1% is added to water to 28 dyn/cm, and reducing interfacial tension from 44 dyn/cm to 8 dyn/cm in a tetradecane/water system, and has the ability to emulsify oils in the presence of hydrophobic surfactants. The HLB of the fenugreek saponin was found to be 18. The extracted saponin stabilizes oil in water emulsions together with Span 80 better than any other tested combinations of emulsifiers. Models constructed on the basis of molecular dynamics exhibit excellent structural fitness.

In Hyomen, Vol. 29, No. 7, pp. 559-570 (1991), as abstracted in CA117:114014x, there is described a review of 81 references on molecular structure, surface activity, and foaming and emulsifying power of soy saponins, effect of carboxy groups on the surface activity of soy saponin, and surface activity of soy saponin methyl ester and soy lipids. In said article there is described the testing of three types of soy saponins, wherein the best lowering of surface tension was achieved with the soy saponin designated SAb, which reduced water surface tension from 72.8 to 40 dyn/cm at 1% concentration.

In contradistinction thereto, the fenugreek saponins of the present invention were found to reduce water surface tension from 72.8 to 28 dyn/cm at 1% concentration.

Similarly, the interfacial tension of SAb reached 10 dyn/cm in a kerosene/water system, while the fenugreek saponins of the present invention reached an interfacial tension of 8 dyn/cm in a tetradecane/water system.

Fenugreek has now also been found to be a preferred source of saponins, since the saponin content, based on dry weight, is 3.6% in chick peas; 4% in lentils; 5.6% in soybeans and over 6% in fenugreek [R.D. Sharma, Nutrition Reports International, "An Evaluation of Hypocholesterolemic Factor of Fenugreek Seeds (T. Foenum Graecum) in Rats," National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India].

The invention will now be described in connection with certain preferred embodiments with reference to the following illustrative figures so that it may be more fully understood.

With specific reference now to the figures in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

Fig. 1 depicts the surface tensions of fenugreek saponins as a function of time (minutes);

Fig. 2 depicts surface tensions of fenugreek saponins as a function of log c;

Fig. 3 shows the interfacial tension of tetradecane/water as a function of saponin concentration;

Fig. 4 shows the percent of oil separation in emulsions prepared with 20 wt.% tetradecane in water in the presence of 5 wt.% saponin-Span 80 blends, at different HLB values, aged for 7 days at 60°C; and

Fig. 5 shows the average droplet size (microns) of emulsions prepared with 20 wt.% tetradecane in water in the presence of 5 wt.% saponin-Span 80 belnds, at different HLB values, aged for 7 days at 60°C.

Example 1

Isolation of the Seed Fractions

Dry seeds of fenugreek were purchased from the local market. The source was T. Foenum graecum, grown in India. The seeds were ground to fine powder (125 g). The extraction was performed with Soxhlet apparatus in the presence of n-hexane for 5 hours, to give a colorless solution. The hexane solution, containing most of the lipids (1.5 g) was discarded. The solid fraction was further treated with ethanol (200 ml), followed by methanol (150 ml). The extract was vacuum evaporated and the solids were lyophilized, to yield 7-8 g of mixture of saponins. These were further treated to isolate the galactomannans, as described and claimed in co-pending Israel Specification No.

Surface and Interfacial Tensions

Surface and interfacial tensions were measured with a Lauda tensiometer and platinum plate, and results were plotted against time and saponin concentrations.

Emulsification

Tetradecane (20 wt.%) was emulsified in the presence of 1-5 wt.% saponin and with blends of saponins and other hydrophobic nonionic surfactants, such as Span 80 or monoglyceride oleate (MGO). The saponin performance in terms of emulsification capacity, stability, required HLB and synergistic effects in the presence of other nonionic emulsifiers, was evaluated. Each emulsion was homogenized, either by homogenizer (Ultra Turrax, Model 25, 8000-24000 rpm) or sheared in the microfluidizer (Microfluidics Corp., Model TC-110).

Droplet size distribution was measured, using a Coulter counter (Electronic Coulter, Model TA-11, U.S.A.).

Modeling

Molecular modeling was performed on a Silicon Graphics computer, using the Insight II program of Biosym Technologies on a work station type Iris 4D/240GTX.

Results

The surface tension of 1 wt.% fenugreek saponin aqueous solution was plotted against time (Fig. 1). The equilibrium time required to obtain constant values is 30 to 40 minutes,

stressing the fact that the saponins are monomeric surfactants with relatively high molecular weights. Each sample was therefore equilibrated for 60 minutes prior to being measured. Fig. 2 shows that fenugreek saponin can reduce surface tension to values lower than 30 dyn/cm (similar to values obtained for sugar esters). The shape of the curve resembles typical behavior of macro molecules.

The interfacial tension of tetradecane/water in the presence of fenugreek saponin was reduced to an impressive value of 8 dyn/cm surface activity, which seems to be better than other nonionic surfactants like ethoxylated sorbitan esters or sugar esters.

Fenugreek saponin characteristics are listed below in Table 1:

TABLE 1

Fenugreek Saponin Characteristics

Solubility in water	Soluble in both cold and hot water
Solubility in ethanol	Soluble in ethanol
Surface tension	28 dyn/cm 1 wt.%
Interfacial tension (with tetradecane	8 dyn/cm 1 wt.%
Efficiency	3
Effectiveness	44 dyn/cm
pH sensitivity	low
HLB	17-18
Emulsion stability	5-6 months
Emulsion average drop size	5 microns

The surface tensions, excess surface concentrations, efficiency, effectiveness, and surface area of the fenugreek saponin have been calculated and compared to other saponins, as presented below in Table 2.

TABLE 2

Properties of Fenugreek Saponin,
Compared to Other Saponins and Selected Monomeric Surfactants

	Surface Tension (dyn/cm)	Excess Surface Concentration (mole/cm^2)	Surface Area (nm)^2	Efficiency	Effectiveness
Fenugreek	28	1.57·E-10	1.05	3	44
Digitonin	42	1.56·E-10	1.06	1.3	30
Sapoalbin	52	1.2·E-10	1.37	≈0	20
Soy Saponin	42	11.0·E-10	4.02	0.7	30

The effectiveness of the saponinic fraction was superior to that of the hydrophilic ethoxylated sorbitan mono oleate (Tween 80), i.e., said fraction had an effectiveness of 44, while that of Tween 80 was only 22.

Span 80 was used as the hydrophobic surfactant to prepare blends with the extracted fenugreek saponin, in order to evaluate its emulsification power and the HLB value. The 20 wt.% tetradecane emulsions were homogenized either by rotary homogenizer or by the microfluidizer. The emulsions' average droplet sizes were monitored against time. Incubation at 60°C, followed by measuring the percentage of oil separation, served as an additional stability tool. Figs. 3 and 4 clearly set the minimum (the best emulsification power) at HLB 17-18.

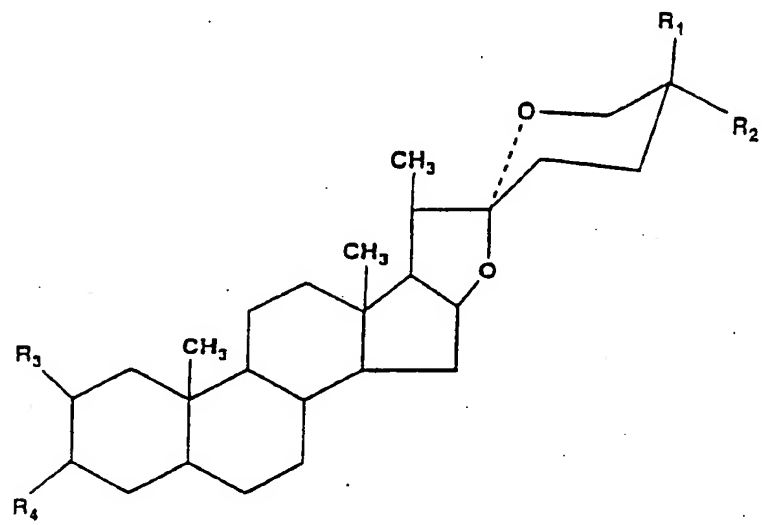
The fenugreek saponin is, therefore, very hydrophilic. The diosgenin represents 41% of the fenugreek saponins and probably dictates its surface properties. It should be noted that the best emulsions were obtained with Span 80. Ethoxylated oleyl alcohol (2EO) and sucrose distearate were non-synergistic to the fenugreek saponins, and led to unstable emulsions.

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is therefore desired that the present embodiments and examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come within the

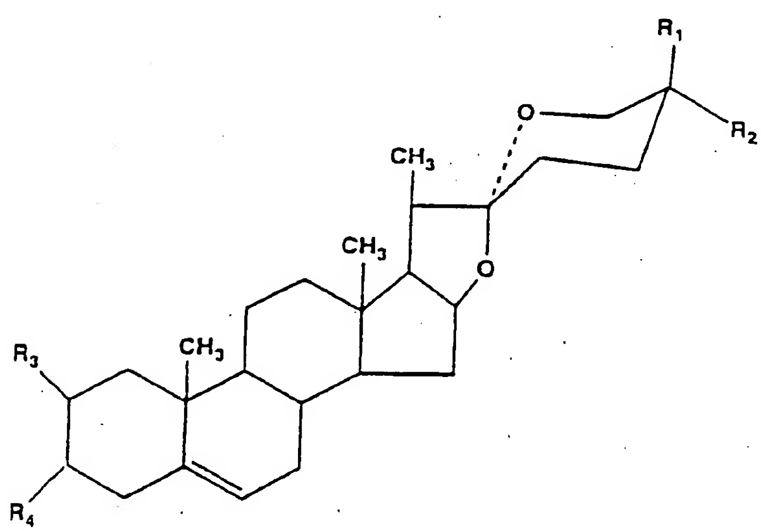
meaning and range of equivalency of the claims are therefore intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A comestible product comprising a fenugreek saponin as an emulsifying agent therein, said fenugreek saponin comprising a mixture of glucosidic steroids having the formula Ia or Ib:



Ia



Ib

wherein:

R_1, R_2 and R_3 are H, CH_3, H; CH_3, H, H; H, CH_3, OH; or CH_3, H, H respectively; and

R_4 is hydrogen or a unit of 2-5 interconnected sugar moieties;

said moieties comprising mannose, glucose, rhamanose, xylose and galactose, and having different and improved properties when compared to products produced with soybean saponins.

2. A comestible product according to claim 1, wherein said fenugreek saponin has the formula Ia and is selected from the group consisting of tigogenin, wherein R_1 is hydrogen; R_2 is methyl; R_3 is hydrogen and R_4 includes 2-glucose, 2-galactose and 1-xylose; neotigogenin, wherein R_1 is methyl; R_2 and R_3 are both hydrogen, and R_4 includes 2-xylose, 1-glucose and 1-rhamanose; gitogenin, wherein R_1 is hydrogen; R_2 is methyl, R_3 is hydroxy and R_4 includes 2-galactose, 1-xyloze and 1-glucose; and neogitogenin, wherein R_1 is methyl and R_2, R_3 and R_4 are each hydrogen.

3. A comestible product according to claim 1, wherein said fenugreek saponin has the formula Ib and is selected from the group consisting of diosgenin, wherein R_1 is hydrogen; R_2 is methyl; R_3 is hydrogen and R_4 includes 1-mannose, 1-glucose, and 1-rhamanose; yamogenin, wherein R_1 is methyl; R_2 and R_3 are both hydrogen, and R_4 includes 2-rhamanose and 2-glucose; yucagenin, wherein R_1 is hydrogen; R_2 is methyl, R_3 is hydroxy; and lilagenin, wherein R_1 is methyl and R_2, R_3 and R_4 are each hydrogen.

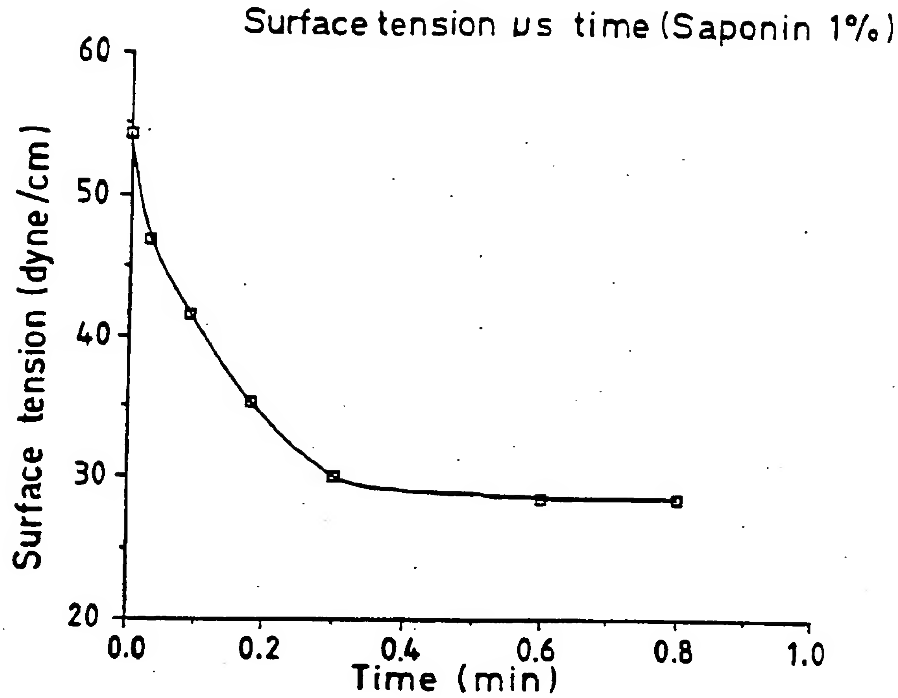

Surface tension vs time (Saponin 1%)
Surface tension (dyne/cm)
60
50
40
30
20
0.0
0.2
0.4
0.6
0.8
1.0
Time (min)

FIG.1

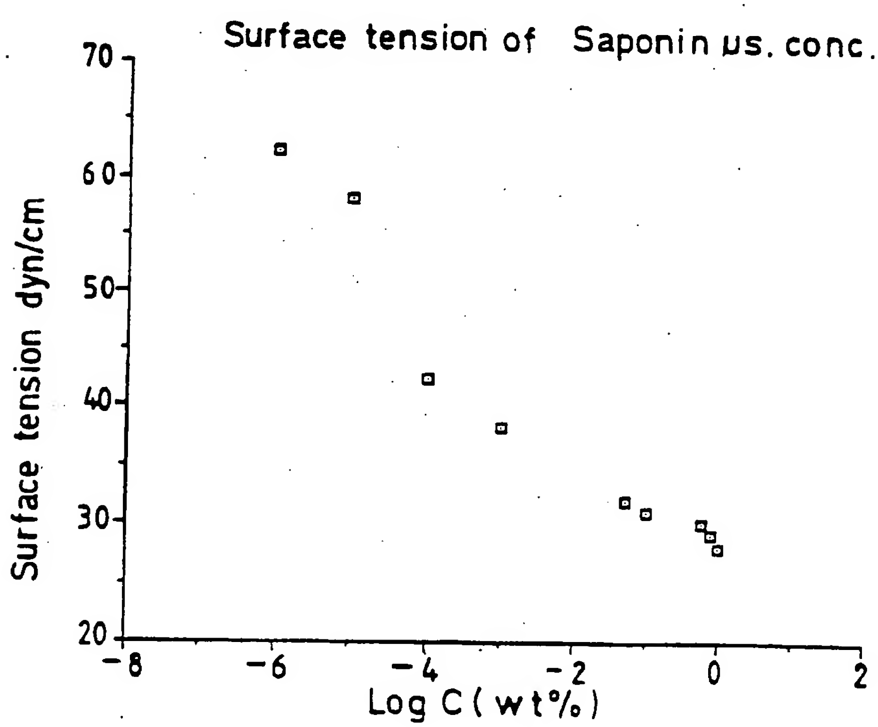

Surface tension of Saponin vs. conc.
Surface tension dyn/cm
70
60
50
40
30
20
−8
−6
−4
−2
0
2
Log C (wt%)

FIG.2

2/3

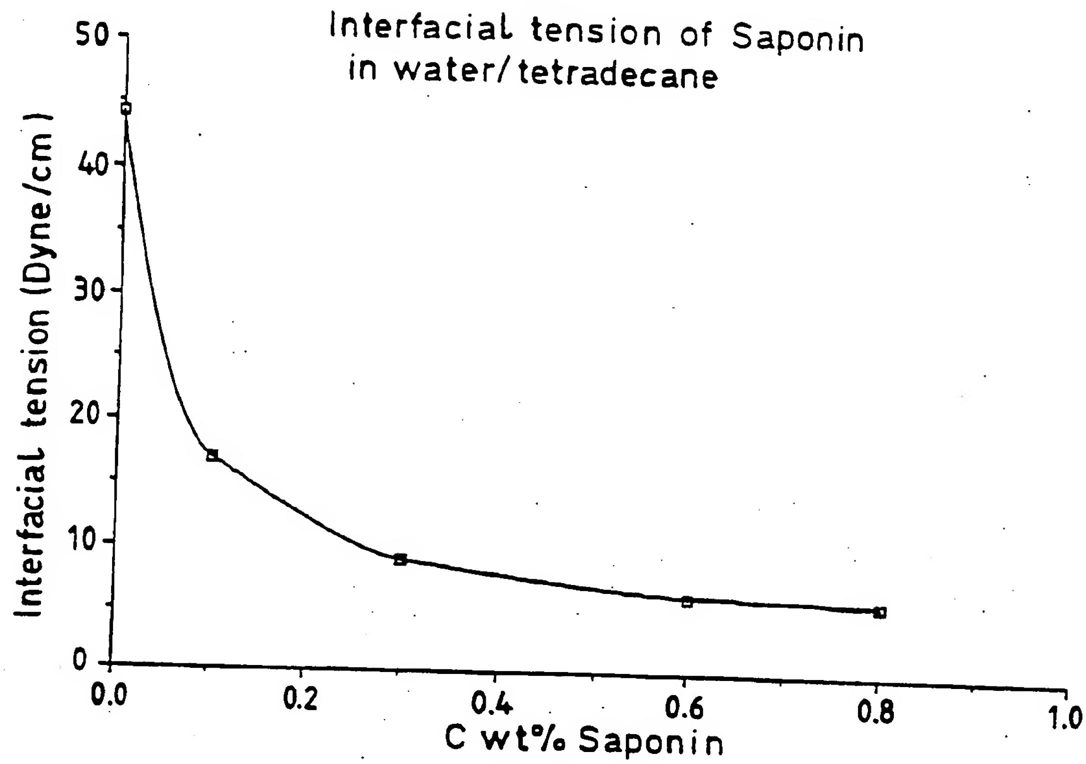

Interfacial tension of Saponin
in water/tetradecane
Interfacial tension (Dyne/cm)
50
40
30
20
10
0
0.0
0.2
0.4
0.6
0.8
1.0
C wt% Saponin

FIG. 3

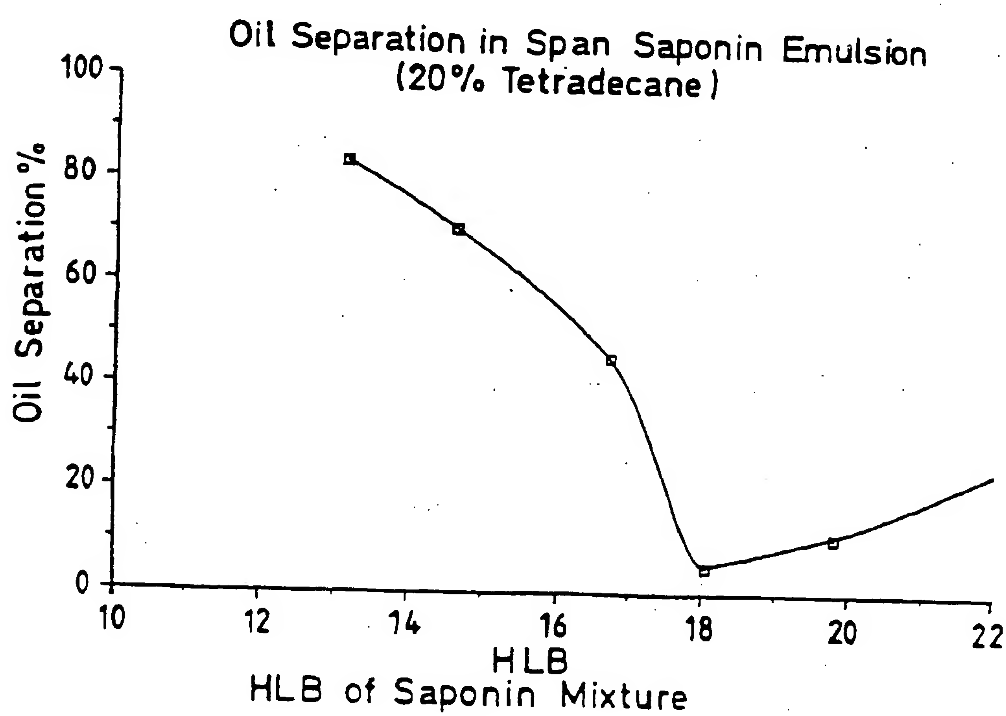

Oil Separation in Span Saponin Emulsion
(20% Tetradecane)
Oil Separation %
100
80
60
40
20
0
10
12
14
16
18
20
22
HLB
HLB of Saponin Mixture

FIG. 4

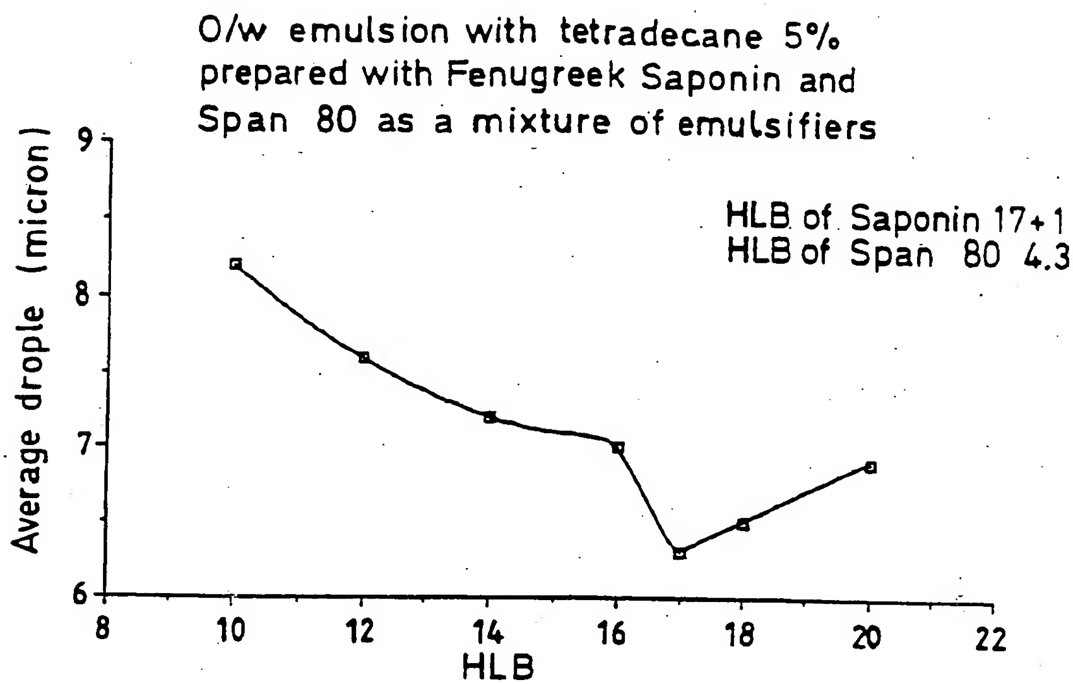

O/w emulsion with tetradecane 5%
prepared with Fenugreek Saponin and
Span 80 as a mixture of emulsifiers
HLB of Saponin 17+1
HLB of Span 80 4.3
Average drople (micron)
9
8
7
6
8
10
12
14
16
18
20
22
HLB

FIG.5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 95/00921

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/035

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FOOD CHEMISTRY, vol. 20, no. 2, 1986 (GB), pages 153-156, EL-SAYED M. ET AL. 'Chemical and Functional Properties of some Legume Powders' see page 155, paragraph 2; table 1	1-3
Y		1-3

X,P	LEBENSM.-WISS.U.TECHNOL., vol. 27, no. 6, 1994 pages 568-572, E.H.MANSOUR AND T.A.EL-ADAWY 'Nutritional Potential and Functional Properties of Heattreated and Germinated Fenugreek Seeds' see table 7	1-3

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
25 August 1995	14.09.95

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